

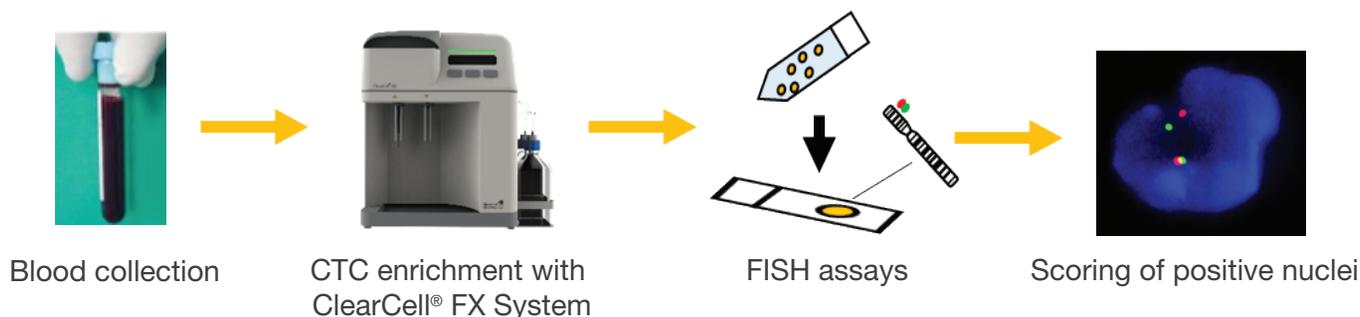
# Fluorescence In Situ Hybridisation (FISH)

## Application Notes

### Overview

Fluorescence in situ hybridisation (FISH) is an established cytogenetic technique used in pathology laboratories for tumour profiling and screening of genetic aberrations, such as gene translocation, amplification and deletions. It is also the current gold standard for selection of cancer patient eligible for HER2 and ALK targeted drug therapy. Here we present the integration of CTC enrichment with ClearCell® FX System and FISH for the detection of ALK gene rearrangement and HER2 gene amplification.

### Workflow



### Results

- 1) The ClearCell® FX System enables retrieval of wholly intact CTCs from cancer blood samples. Cells are retrieved in their native state and can be coupled with FISH assays.
- 2) Metastatic cancer patients who are tested positive for ALK mutation and HER2 amplification on primary tumours were concomitantly found to have circulating tumour cells harbouring the respective mutations (Figures 1A, 1B).
- 3) Enriched CTC samples are immunolabeled with pan-cytokeratin antibodies and FISH probes to illustrate the feasibility of coupling immunofluorescent staining with FISH on CTC samples derived from lung cancer patients (Figure 1c).

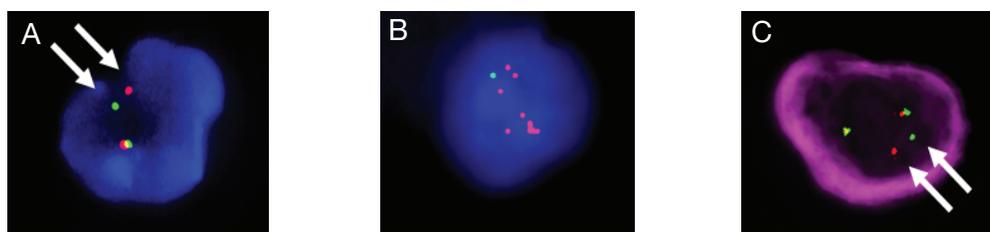


Figure 1. Representative images showing detection of chromosomal abnormalities with FISH. (A) ALK positive CTCs from lung cancer patients are analysed with Vysis ALK break apart FISH probes (Green and Orange dots) and counterstained with DAPI. White arrows indicate the positions of break apart signals. (B) HER2 gene amplification in CTCs derived from breast cancer patients (Lab Chip, 2013). Cells are scored as positive when the HER2/CEN17 ratio (Orange/Green) is greater than 2. (C) Enriched CTC sample was immunolabeled with pan-cytokeratin antibodies (Magenta) prior to ALK FISH for concurrent identification of cytokeratin-expressing cells (potential CTCs) and analysis of FISH results (Images courtesy of Singapore General Hospital and National Cancer Centre Singapore).

### Conclusion

ClearCell® FX System offers a label free means to enrich CTCs from whole blood and enables the integration of CTC with FISH. CTC molecular profiling with FISH offers a glimpse into the molecular profile of the original tumour, and could potentially serve as a surrogate for obtaining clinically useful information. Coupled with immunostaining, CTC “immuno-FISH” offers a means for more efficient molecular characterisation of CTCs.